CLAIM LISTING

[~~Claims :~~]

We claim

Claims 1-23 : canceled.

24.(Currently amended) A[~~n automated~~] method of one step simultaneous synthesis of labeled internal standards and additional one step chemical reaction for the purpose of determination of metabolic stability of a library of chemical compounds from the in vitro cytochrome P450 enzyme reactions by [~~isotope dilution~~] mass spectrometric (MS) analysis comprising the steps of :

a) synthesizing labeled internal standards of all library compounds simultaneously by a one step reaction of an authentic sample of all library compounds with a labeled derivatizing reagent having at least one enriched stable isotope atom to form derivatized chemical compounds having a mass increase by the chemical attachment of labeled tagged chemical group [~~converting a portion of each said library compound into a stable isotope labeled internal standard by addition of a stable isotope labeled chemical reagent~~]; and

b) isolating said labeled internal standards from said one step reaction by an aqueous extraction method [~~converting another portion of each said library compound into a compound of identical structure, with the exception of the~~

~~stable isotope atoms, as that of said stable isotope labeled internal standard comprising addition of a non-isotope version of said stable isotope reagent~~]; and

c) contacting library compounds with cytochrome P450 enzymes in reactors at 37 degree Celcius for different time intervals [~~separating said stable isotope labeled internal standards and said non-isotope labeled converted compounds by an extraction method~~]; and

d) stopping enzyme reactions at different time intervals by addition of a solution containing an organic solvent and a known quantity of said labeled internal standards [~~incubating other portions of said library of compounds in the presence of cytochrome P450 enzyme systems at body temperature for periods of time~~]; and

e) contacting the remainder of said library compounds in stopped enzyme reactions with a non labeled version of said derivatizing reagent used in step a) to form derivatized library compounds having the same but non labeled tagged chemical groups as those of said labeled internal standards and wherein said labeled internal standards are not reacted with said non labeled derivatizing reagent [~~adding quenching chemical reagents to terminate said enzyme reactions~~]; and

f) isolating said derivatized library compounds and said labeled internal standards from said stopped enzyme reactions by an aqueous extraction method [~~adding said stable isotope labeled internal standards to said terminated reactions~~]; and

g) analyzing said derivatized library compounds and said labeled internal standards in said aqueous extract by mass spectrometry to determine the concentration of the remainder of said library compounds in stopped enzyme reactions and to calculate their remaining percentages [~~adding said non-isotope version of said stable isotope labeled chemical reagent to said terminated reactions to convert the remaining of each said library compound into said non-isotope labeled converted compounds~~]; and

[~~separating said converted compound and its internal standard from said reactions by an extraction method; and~~]

i) [~~determining the molecular ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and~~]

j) [~~determining the most abundant daughter ions of said stable isotope labeled internal standards and said non-isotope labeled converted compounds; and~~]

k) [~~determining the ion ratio of said converted compound to said corresponding internal standard for each of said enzyme reactions using tandem mode of mass spetrometric analysis; and~~]

l) [~~determining the percent remaining of each said library compound from said enzyme reactions from said ion ratios.~~]

25. (Canceled).

26. (Currently amended) The method of claim 24 wherein said aqueous extraction in steps [~~c~~] b) and [~~h~~] f) can be any appropriate [~~separating~~] extraction method[~~s~~] such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

27. (Currently amended) The method of claim 24 wherein [~~said sample contains either a singularity or a plurality of each class of said~~] library compounds all having in common at least one functional group, including amino group represented as R_2NH , hydroxyl group represented as R_3COH, carbonyl group represented as R_2CO , and carboxyl group represented as RCOOH, wherein R is a part of the library compounds composed of hydrogen, alkyl, aryl, cyclic, non-cyclic~~,~~ hetero atom containing groups.

28. (Currently amended) The method of claim 24 wherein said [~~library compounds in step a) are converted to said~~] labeled internal standards are synthesized

simultaneously from reaction with [~~using~~] a single stable isotope labeled [~~chemical~~] derivatizing reagent having the ability to chemically attach the same labeled tagged chemical group to common functional groups of all library compounds.

29. (Currently amended) The method of claim 24 wherein said derivatized library compounds in step[~~s b) and g)~~] e) [~~are converted to compounds of~~] have identical structure as that of the corresponding member of said labeled internal standards, except the labeled atoms[~~, using a single non-labeled version of said chemical reagent~~].

30. (Currently amended) The method of claim 24 wherein [~~said conversion~~] the one step simultaneous reaction in steps a), [~~b)~~] and [~~g~~] the additional one step reaction of step d) are 100% quantitative meaning the remainder of said library compounds are completely derivatized.

31. (Canceled).

32. (Currently amended) The method of claim 24 wherein said [~~quenching chemical reagent is an~~] organic solvent used to stop the enzyme reactions includes methanol, acetonitrile, ethyl acetate.

33. (Currently amended) The method of claim 24 wherein said labeled derivatizing reagent includes labeled acid anhydride $(R*CO)_2O$ or labeled acid chloride $R*COCl$,

labeled chloroformate R*OCOCl , labeled isocyanate R*NCO, and labeled thioisocyanate R*NCS , to react with amino and hydroxyl groups and the derivatized compounds all having a mass increase by the tagged chemical group R*CO, R*OCO , R*NHCO , and R*NHCS , respectively, wherein R* is labeled alkyl or labeled aryl groups containing at least one enriched stable isotope atom [~~said library compounds are all primary and/or secondary amines and said stable isotope reagent is selected from a group consisting of a labeled acid anhydride or labeled acid chloride, labeled chloroformate, labeled isocyanate, and labeled thioisocyanate, and said resulting internal standards are labeled amides, labeled carbamates, labeled ureas, and labeled thioureas, respectively~~].

34. (Canceled).

35. (Currently amended) The method of claim 24 wherein said labeled derivatizing reagent includes R*ONH$_2$ and R*NHNH$_2$, to react with carbonyl group and the derivatized compounds all having a mass increase by the tagged chemical group R*ON and R*NHN , respectively, wherein R* is labeled alkyl or labeled aryl groups containing at least one enriched stable isotope atom [~~in which said library compounds are all aldehydes and/or ketones and said stable isotope reagent is selected from a group consisting of a labeled alkoxylamine and a labeled alkylhydrazine, and said resulting internal standards are labeled oximes and labeled hydrazones, respectively~~].

36. (Currently amended) The method of claim 24 wherein said labeled derivatizing reagent includes R*OH , R*Cl , R*Br , R*I , to react with carboxyl group and the derivatized compounds all having a mass increase by the tagged chemical group R*, wherein R* is a labeled alkyl group containing at least one enriched stable isotope atom [~~in which said library compounds are all carboxylic acids and said stable isotope reagent is either a labeled alcohol and a chloroformate or a labeled alkyl halide and a base and said resulting internal standards are labeled carboxylic acid esters~~].

37. (Original) The method of claim 24 wherein said cytochrome P450 enzyme systems are cryopreserved or fresh human and animal hepatocytes, microsomes, S9 fractions or solutions containing individual cytochrome P450 isoenzymes.

38. (Currently amended) The method of claim 24 wherein said non labeled derivatizing reagent includes acid anhydride $(RCO)_2O$ or acid chloride RCOCl , chloroformate ROCOCl , isocyanate RNCO , and thioisocyanate RNCS to react with amino and hydroxyl groups and the derivatized compounds all having a mass increase by the tagged chemical group RCO , ROCO , RNHCO , and RNHCS , respectively, wherein R is alkyl or aryl groups [~~said library compounds are all primary or secondary amines and said non-labeled version of said stable isotope labeled reagent in steps b and g is selected from a group consisting of an acid anhydride or acid chloride, a chloroformate, an isocyanate, and a thioisocyanate and said resulting converted compounds are amides, carbamates, ureas and thioureas, respectively~~].

39. (Canceled).

40. Currently amended) The method of claim 24 wherein said non labeled derivatizing reagent includes $RONH_2$ and $RNHNH_2$, to react with carbonyl group and the derivatized compounds all having a mass increase by the tagged chemical group RON and RNHN , respectively, wherein R is alkyl or aryl groups [~~said library compounds are all aldehydes and/or ketones and said non-labeled version of said stable isotope labeled reagent in steps b and g is selected from a group consisting of an alkoxylamine and an alkylhydrazine, and said resulting converted compounds are oximes and hydrazones, respectively~~].

41. (Currently amended) The method of claim 24 wherein said non labeled derivatizing reagent includes ROH , RCl , RBr , RI, to react with carboxyl group and the derivatized compounds all having a mass increase by the tagged chemical group R, wherein R is an alkyl group [~~said library compounds are all carboxylic acids and said non-labeled version of said stable isotope labeled reagent in steps b and g is either an alcohol and a chloroformate or an alkyl halide and a base, and said resulting converted compounds are esters~~].

42. (New) The method of claim 24 wherein said enriched stable isotope atom includes deuterium, carbon-13, nitrogen-15, and oxygen-18.

43. (New) The method of claim 24 wherein said labeled derivatizing reagent is acetic acid anhydride-d6 $(CD_3CO)_2O$ to react with amino and hydroxyl groups and the

derivatized compounds all having the tagged chemical group CD_3CO resulting in a mass increase of 45amu.

44. (New) The method of claim 24 wherein said non labeled derivatizing reagent is acetic acid anhydride $(CH_3CO)_2O$ to react with amino and hydroxyl groups and the derivatized compounds all having the tagged chemical group CH_3CO resulting in a mass increase of 42 amu.